Isolation and identification of *Acanthamoeba* genotypes and *Naegleria* spp. from the water samples of public swimming pools in Qazvin, Iran

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ABSTRACT

Free-living amoeba (FLA), including *Acanthamoeba* and *Naegleria* are facultative parasites in humans. The amoeba have widespread distribution in various water sources. The aim of this study was isolation and molecular identification of *Acanthamoeba* and *Naegleria* isolated from swimming pools and also hot and cold tub waters in Qazvin province. The samples (166 water samples) were cultured to isolate and identify positive specimens. PCR (polymerase chain reaction) amplification, sequencing and phylogenetic analysis were conducted to confirm the isolated species and genotypes of amoeba. According to morphological characterizations, 18.6% of specimens were identified as FLA, which in 71% were *Acanthamoeba* by PCR method. Molecular analysis revealed that 36.3%, 18.1% and 4.5% of *Acanthamoeba* specimens were identified as T3, T4 and T11 *Acanthamoeba* genotypes, respectively. *Protacanthamoeba bohemica* (27.2%) and *Acanthamoeba* sp. (4.5%) were found among the specimens. The results of osmo-tolerance and thermo-tolerance assays demonstrated that 50% of T3 and 25% of T4 genotypes of *Acanthamoeba* were highly pathogenic parasites. The molecular approach showed the presence of *Naegleria lovaniensis* (9%) in hot tub water of swimming pools. This study demonstrated that the swimming pools and hot tub water in Qazvin province were contaminated with *Acanthamoeba* and *Naegleria* species.

Key words | *Acanthamoeba*, free-living amoeba, genotype, Iran, *Naegleria*

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INTRODUCTION

Free-living amoeba (FLA) are opportunistic protozoan parasites and some FLA, such as *Acanthamoeba*, *Naegleria*, *Balamuthia* and *Sappinia* are considered as pathogenic amoeba (Visvesvara *et al.* 2007; Henriquez & Khan 2009; Yousuf *et al.* 2013). The FLA can cause severe and even fatal diseases by invasion and causing damage to the central nervous system (CNS) and other organs. A wide range of diseases including amoebic encephalitis, keratitis and skin ulcerations have been reported in both immunocompetent and immune-deficient individuals (Khan 2006; Henriquez & Khan 2009). Moreover, the number of cases of amoebic keratitis (AK) in Iran has increased in the recent decade which is probably due to poor hygiene among contact lens users (Rezaeian *et al.* 2007). The amoeba can be the reservoir and vehicle of the microbial world, in particular pathogenic bacteria present in nature as they can transfer such microorganisms to humans, leading to enhanced amoeba infection (Rezaeian *et al.* 2008; Siddiqui & Khan 2012; Buse *et al.* 2016).

Amoebae have widespread distribution in different environments including water, soil, dust and air sources. Various water sources, such as pool, river and recreational waters can be contaminated with FLA. Thermal water was described as a suitable niche for *Naegleria* spp., therefore,

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